



The biocatalytic conversion of 8-hydroxymanzamine A to manzamine A

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Abstract—The selective dehydroxylation of 8-hydroxymanzamine A (**1**) to form manzamine A (**2**) can be completed by fermentation with *Fusarium solani* or *Streptomyces seokies*. This unique biocatalytic conversion is important due to the fact that manzamine A has more desirable biological activity when assayed in a murine model against malaria. © 2003 Elsevier Science Ltd. All rights reserved.

8-Hydroxymanzamine A (8-OHMA) (**1**) is one of the common manzamine alkaloids that has been isolated from sponges of the genera *Amphimedon*, *Xestospongia* and *Pachypellina*.^{1,2} We examined the biotransformation of 8-OHMA in order to identify products with improved activity or reduced toxicity as anti-infective agents.

Several biocatalytic studies of marine natural products have been recently reported indicating that biocatalysis can produce modified marine products with enhanced biological activity and reduced toxicity.^{3,4} We report here the conversion of 8-OHMA (**1**) to manzamine A (MA) (**2**) by *Fusarium solani* and *Streptomyces seokies* with a focus on improved yields for this transformation.

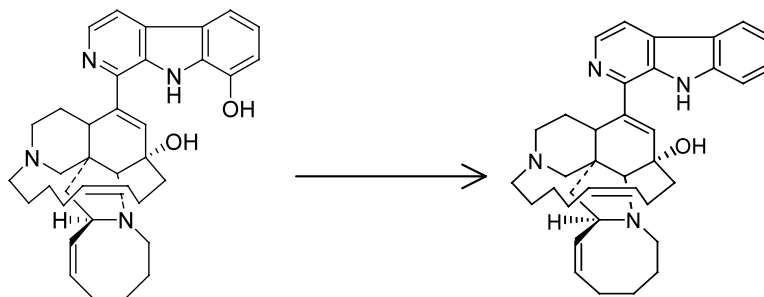
Eight *Fusarium* species⁵ were screened for their ability to transform 8-OHMA. Preliminary screening was completed using media containing (g/L) glucose (20), peptone (5), yeast extract (5) and KH₂PO₄ (5). The 8-OHMA utilized for this study was isolated by our group from several of the common Indo-Pacific manzamine-producing sponges collected near Manado, Indonesia. The purity of 8-OHMA was confirmed by

HPLC and NMR before utilizing for biotransformation studies.⁶ Ten mg of 8-OHMA (**1**) was dissolved in 1 mL acetone and then 100 µL of this solution was added to 25 mL of media inoculated with 1×10⁵ conidia of *Fusarium* sp. Culture controls consisted of medium and each *Fusarium* species was grown under identical conditions without 8-OHMA. A substrate control was composed of medium and 8-OHMA without the microorganism. Cultures were shaken on a gyratory shaker at 150 rpm and 25°C for 7 days. The cultures were separated by vacuum filtration into mycelia and media. The media and mycelia were extracted with 100 mL chloroform and the extracts were monitored by TLC (silica gel GF, hexane–acetone: 7:3, Dragendorff solution). A single biotransformation product with an *R_f* value identical to MA was identified from the culture of *F. solani*. Based on the production of the biotransformation products, *F. solani* was selected for scale-up.

Scale-up was carried out using *F. solani* and *S. seokies*. Three concentrations of *F. solani* conidia 1×10⁵, 1×10⁶ and 1×10⁷ were evaluated for suitable biotransformation. Each flask containing 200 mL media and 50 mg of 8-OHMA was inoculated with one of 3 conidial concentrations. Culture conditions were the same as those previously described and the transformation of 8-OHMA was monitored by TLC during fermentation to determine an end point. Cultures were harvested after 20 days, and then filtered, and extracted with chloroform. A single metabolite was identified from a culture

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8-hydroxymanzamine A (1)

manzamine A (2)

with 1×10^6 conidia. Purification by HPLC⁷ afforded 4 mg of metabolite from the medium. Proton NMR and mass spectra showed that this metabolite was MA.

A biotransformation study using *Streptomyces seokies*⁸ was completed in order to improve the yield. *S. seokies* was selected based on our screening study that indicated the culture successfully metabolize both manzamine A and E. 25 mL of media was inoculated with 1.5 mL 30% PCV (Packed Cell Volume) of *S. seokies* and fed 1 mg of 8-OHMA. At the end of the exponential growth phase (4 days) the culture was harvested. Mycelia and medium were separated through vacuum filtration and extracted with chloroform. The extract was monitored by TLC and LC-MS was utilized to confirm that the metabolite was MA based on retention time and molecular weight. The reaction was then scaled-up to determine the yields.

The scale up was completed using the following conditions: 200 mL of media was inoculated with 10 mL of 30% PCV of cultured *S. seokies* 2 days old (exponential growth phase). 8-OHMA (75 mg) was dissolved in 0.5 mL acetone and added to the culture. The culture was shaken on a gyratory shaker 150 rpm at 25°C and the culture was monitored by TLC to identify the accumulation of MA, and harvested after 9 days. Mycelia and media were separated and extracted with chloroform. Extraction afforded 52 mg of extract from media and 22 mg of extract from the mycelia. Purification by HPLC afforded 30 mg of pure metabolite from the medium and 10 mg pure metabolite from the mycelia. Proton NMR and mass spectra confirmed that the metabolite from the medium is MA and the metabolite from the cells is 8-OHMA.

Based on these results it is clear that both *S. seokies* and *F. solani* can transform 8-OHMA to MA with yields of 40 and 8%, respectively. This biocatalytic reaction is unique and valuable due to the fact that the transformation involves a selective dehydroxylation of the C-8 position of 8-OHMA. This type of selectivity in an alkaloid as sophisticated as the manzamines would be clearly difficult to achieve without the use of an enzyme-based approach. In addition, this biotransformation produces a product of greater value due to the improved activity of MA as an antimalarial agent over the readily available 8-hydroxy analog.^{9,10}

Given sufficient time we found that both microbes were able to completely metabolize all available MA. This indicates that the transformed MA can be utilized as a primary metabolite for growth by these two species. The mechanism for the complete metabolism of MA will yield important clues to potential mechanisms of resistance to this promising broad-spectrum anti-infective agent. Additional details regarding the toxicology, metabolism and biotransformations of these compounds will be published in due course.

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7. HPLC: column 250×10 mm, 5 μ Luna C8 Phenomenex, mobile phase: water and acetonitrile with 0.1% TFA. Isocratic elution for first 10 min with 80% water followed by gradient elution at constant flow rate 2 mL/min.
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